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Adaptation of *Saccharomyces cerevisiae* to high hydrostatic pressure causing growth inhibition

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Abstract Genome-wide mRNA expression profiles of *Saccharomyces cerevisiae* growing under hydrostatic pressure were characterized. We selected a hydrostatic pressure of 30 MPa at 25 °C because yeast cells were able to grow under these conditions, while cell size and complexity were increased after decompression. Functional characterization of pressure-induced genes suggests that genes involved in protein metabolism and membrane metabolism were induced. The response to 30 MPa was significantly different from that observed under lethal conditions because protein degradation was not activated under 30 MPa pressure. Strongly induced genes those that contribute to membrane metabolism and which are also induced by detergents, oils, and membrane stabilizers.

Keywords: Yeast; Hydrostatic pressure; DNA microarray; *Saccharomyces cerevisiae*

1. Introduction

Hydrostatic pressure is a physical factor that can cause stress to organisms [1]. This stress causes cellular death, cellular arrest and growth inhibition, and these effects depend on the degree of pressure, temperature, and sensitivity of the organisms to pressure [2]. For example, *Lactobacillus casei* is killed under 300 MPa [3] and 550 MPa is lethal to *Escherichia coli* [3]. On the other hand, eucaryotic microorganisms are more sensitive to pressure. *Saccharomyces cerevisiae* is sensitive to pressures of more than 150 MPa [4] and *Penicillium roqueforti* was shown to be more sensitive than *Escherichia coli*, which was more sensitive than *Staphylococcus aureus* [5]. Interestingly, the effect of pressure strongly depends on temperature. Sonoike et al. [3] showed that *L. casei* is killed under 300 MPa with a death rate of 0.32 at 0 °C, 0.1 at 20 °C, and 0.32 at 60 °C. For yeast cells, 40 MPa is lethal at 4 °C, but at 25 °C and 40 MPa, cells are able to grow [6].

The mechanisms by which the growth and viability of organisms are affected by pressure (the field of ''piezophysiology'') are being intensively studied [2]. The field of piezophysiology has been reviewed [2], but a brief introduction follows. In studying the range of pressures that causes growth inhibition, Abe and Horikoshi [7] found that hydrostatic pressures of 40–60 MPa promoted acidification of the vacuoles in yeast cells and that expression of the tryptophan permease gene *TAT2* can be the rate limiting factor affecting the growth of tryptophan-requiring yeast cells [8]. Tamura et al. [9] showed the induction of a gene for the yeast heat-shock protein in the same range of pressures. In the range of pressure that causes cellular death, Iwahashi et al. [4] showed that treatment with a pressure of more than 150 MPa decreased the CFU but a mild heat-shock treatment of 43 °C for 30 min increased barotolerance (resistance to hydrostatic pressure). Hamada et al. [10] observed the induction of tetraploids or homozygous diploids in the industrial yeast *S. cerevisiae* by hydrostatic pressure (above 100 MPa). Using immunoelectron microscopy with thin frozen sections, Kobori et al. [11] demonstrated that the same range of pressures caused damage to the nuclei of *S. cerevisiae*.

Recently, we studied the lethal effect of pressure on yeast cells. We used yeast DNA microarrays and analyzed the expression levels of 6000 genes. The genome-wide expression profiles suggested that high pressure caused damage to cellular organelles and the damage was similar to that caused by detergents, oils, and freezing/thawing [6]. However, genome-wide expression profiles under pressures that cause growth inhibition has not yet been carried out. According to previous studies [2] in the field of piezophysiology, the effects of pressures that cause growth inhibition must be different from those caused by lethal pressures.

In this report, we focused on genome-wide mRNA expression profiles of *S. cerevisiae* grown under 30 MPa of pressure, which was shown to cause growth inhibition. We found that genes involved in membrane metabolism were significantly activated and the response was essentially different from the response to pressures that cause cellular death.

2. Materials and methods

2.1. Strains and growth conditions

Saccharomyces cerevisiae strain S288C (*mat alpha SUC2 mal mel gal2 CUP1*) was grown in YPD medium (2% polypeptone, 1% yeast extract, and 2% glucose) at 25 °C in pre-cultures for 2–3 days. This strain was used because the probes on the DNA microarray were produced from S288C as the template for PCR.

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2.2. Pressure treatment

To achieve an environment with a specific pressure, a 30-11HF4 high pressure vessel (High Pressure Equipment Co. Ltd., USA) with a volume of 500 ml and with a limit of 100 MPa was used.

For pressure treatment, the pre-culture was inoculated into a 50 ml syringe containing YPD medium with a dilution rate of 1000 for the control and 100 for the pressure treatment. The differential dilution rates were used to obtain cells in the same growth phase after treatment for 16 h. These syringes were set in the high pressure equipment, pressurized and incubated at 25 °C. The control was treated in the same way except for the pressure and dilution rate. After incubation for 16 h, cells were used for DNA microarray analysis or flow cytometry analysis.

2.3. Flow cytometry analysis

Flow cytometry analysis was carried out with an EPICS XLTM flow cytometer (Beckman Coulter Inc., Hialeah, FL, USA) equipped with a 15 mW argon-ion laser (excitation wavelength, 488 nm). Cells in YPD medium (Table 1) or diluted with YPD medium (Fig. 1) were directly applied. Data were analysed using the computer program windows multiple document interface flow cytometry application (WinMDI; J. Trotter, Salk Institute for Biological Studies, San Diego, CA, USA).

2.4. DNA microarray analysis

DNA microarray analysis was carried out with four independent cultures. Total RNA was isolated by the hot-phenol method. Poly(A)$^+$ RNA was purified from total RNA with Oligotex-dT30 mRNA purification kits (Takara, Kyoto, Japan). Two to four micrograms of poly(A)$^+$ RNA was used for each labeling experiment, and the same amount of each poly(A)$^+$ RNA was used on each slide. The two labeled cDNA pools were mixed and hybridized with a yeast DNA chip (DNA Chip Research, Inc., Yokohama, Japan) for 24–36 h at 65 °C. On this microarray, ORFs of 200–8000 bp DNA (0.1–0.5 ng) were spotted and 5880 genes could be analyzed under these conditions [12]. The details of the microarray procedure and validation studies with our conditions have been described previously [12–14]. Detected signals for each ORF were normalized by the intensity dependent (LOWESS) methods (http://www.silicongenetics.com/cgi/SiG.cgi/index.smf). Genes called as induced or repressed were those passing a one sample *t* test (*P* value cutoff 0.05) and additionally showing more than 2-fold higher or lower expression, respectively, compared to the control. The selected genes were characterized according to the categories of MIPS (Munich Information Center for Protein Sequences, http://mips.gsf.de/). The data obtained in this experiment are available with the accession number of GSE2526 in the Gene Expression Omnibus Database (GEO, http://www.ncbi.nlm.nih.gov/geo/).

2.5. RT-PCR

Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out to confirm the result of microarray experiments. The gene name (systematic name) and forward and reverse primer sequence are as follows:

PST1 (YDR055W) 5′-TGCTGCTTCTGCCTCTAGTGTT-3′ and 5′-CACATGTCGTGAATGACAGGTACT-3′, *INO1* (YJL153C) 5′-GGTGGACCCAGTTAAAGAAGATG-3′ and 5′-TGGAAGGGATAATAGGATAATGGTG-3′, *RTA1* (YGR213C) 5′-GAAGAGTCTATTCAAGCGCAACA-3′ and 5′-GAACCCCACTGGCAATATATGAA-3′, *OPI3* (YJR073C) 5′-GCTGGTTGTTTCTGCCGTAGTT-3′ and 5′-CGCTAGATGCTCTCATTGTATTCCT-3′, *POX1* (YGL205W) 5′-CTCCTATAGGTTACTTTGATGGCGATA-3′ and 5′-AAAGTCGCAAAACAGAGGGTTC-3′, *PRM5* (YIL117C) 5′-CAGTCCAAAAGAAAACCTACACCTT-3′ and 5′-ACGCACACACAAAAGAATAGAACC-3′, *SED1* (YDR077W) 5′-AATCTAAGGGCACTACCACCAAAG-3′ and 5′-AGCATTAAGAAGGCGGATGTGT-3′, *ACT1* (YFL039C) 5′-ATTGCCGAAAGAATGCAAAAGG-3′ and 5′-CGCACAAAAGCAGAGATTAGAAACA-3′.

Total RNA was extracted from cells grown under 30 MPa for 16 h at 25 °C or under 0.1 MPa. RT-PCR was performed using the One Step RNA PCR Kit (TaKaRa, Japan). Temperature and cycle conditions were as follows: 70 °C for 3 min, 50 °C for 30 min, 92 °C for 2 min, 20–30 cycles of (94 °C 30 s, 55 °C 30 s, 72 °C 45 s), and 72 °C for 10 min.

Table 1
FACS analysis of pressure treatment

Pressure (MPa)	Counts/min, thousands		FS		SS	
	Mean	S.D.*	Mean	S.D.	Mean	S.D.
0.1	354	118	33	3.6	31	1.5
10	189	72	38	0.6	33	3.8
20	77	28	38	2.6	38	2.0
30	13	5	62	3.8	68	6.4
40	9	2.9	57	2.1	56	5.3

Yeast cells were treated with the indicated pressures and analyzed by FACS.
The relative number of cells is measured as counts per minute. Forward-scattered (FS) values and side-scattered light (SS) values were also determined from the average value not from peak value. Values are mean and S.D. from at least three independent experiments.
*S.D.: standard deviation.

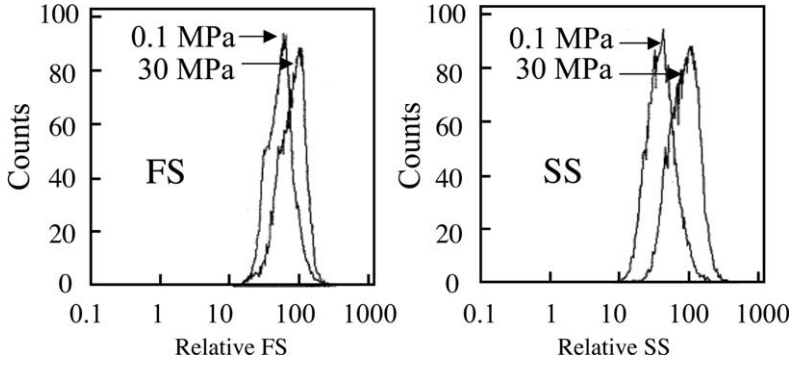

Fig. 1. Flow cytometry analysis of yeast cells grown under 30 MPa pressure. Flow cytometry analysis was carried out with an EPICS XLTM flow cytometer equipped with a 15 mW argon-ion laser and an excitation wavelength of 488 nm. Cells in YPD medium or diluted in YPD medium were directly applied. One of three independent experiments is shown, all of which gave similar results.

3. Results

3.1. Conditions for pressure treatment

The purpose of these studies was to understand the adaptation mechanisms of yeast cells to high hydrostatic pressure conditions using DNA microarray analysis. For DNA microarray analysis, appropriate experimental conditions must be selected. For example, the IC_{50} can be used as the condition for toxicological analysis [15], and LD_{50} for studying freeze/thaw stress [16] or lethal pressure stress [6]. Without biological or physiological characterization of the treatment, we cannot prove that the induction or repression of specific genes is due to the treatment. Lack of growth inhibition would merely show that the condition studied did not cause cell stress and the results obtained did not necessarily reflect the stress.

To find appropriate conditions, we attempted to determine the IC_{50} caused by pressure. During this procedure, we found that cell density (absorbance at 660 nm) was much higher than the corresponding CFUs (colony forming units) after pressure treatment (data not shown). This suggests that cells become bigger after pressure treatment. Thus, we subjected yeast cells grown under various pressure conditions for 16 h to flow cytometry analysis after decompression (Fig. 1 and Table 1). In Fig. 1, yeast cells in YPD medium were applied after dilution with fresh medium to give the same order of cell numbers for both conditions. The peaks of FS (forward scattered) values and SS (side scattered) values were higher after the pressure treatment (Fig. 1). The FS value comes from forward scattered light and corresponds to the relative cellular size, whereas the SS value comes from side scattered light and corresponds to the relative complexity of inner cellular structure [17]. Table 1 summarizes the pressure conditions, counts (cell numbers), and averages of FS and SS values. According to the number of counts, 10 MPa corresponds to IC_{50}, while the cells grown under 30 and 40 MPa showed dramatically different FS and SS values from those of cells grown under 0.1 MPa. Therefore, we can either select 10 or 30 MPa as an appropriate test condition. We selected 30 MPa for our initial studies, because the biological effects of the higher pressure treatment may result in significant differential mRNA expression. The different cellular size and cellular complexity after decompression may result from different expression profiles under high pressure compared to that under atmospheric pressure.

3.2. Characterization of expression profiles of cells grown under 30 MPa pressure

We compared the expression profiles of yeast cells grown under a pressure of 30 MPa to those of yeast cells grown under 0.1 MPa using four sets of DNA microarrays in four independent experiments. From 5721 ORFs that showed intensities over the cutoff value [13], 366 genes showed more than 2-fold higher intensities and 253 genes showed lower than 0.5-fold intensities. The highly induced genes are listed in Table 2. The most highly induced genes were *PST1*, followed by YLR194C, *INO1*, YHR209W, and YCR 007 C. These genes were induced by more than 15-fold.

The 366 induced genes were characterized using the functional categories of MIPS (Table 3). High numbers of genes were induced in the categories of "Cell cycle and DNA processing", "Cell rescue, defense and virulence", and "Metabolism" (Table 2). In addition, a large proportion of genes in the category of "Cell rescue, defense and virulence" (13.7%) was observed. It is significant that genes in subcategories of "stress response" "amino acid metabolism", "nitrogen and sulfur metabolism", "C-compound and carbohydrate metabolism", "cell growth/morphogenesis", and "lipid, fatty-acid and isoprenoid metabolism" were induced (Table 3). Based on the microarray results, it appears that 30 MPa pressure causes yeast cells to activate the stress response as well as pathways for the metabolism of C-compounds, lipids, and amino acids.

Table 2
Genes highly induced after growth under 30 MPa pressure

Systematic number	Fold	S.D.	Common name	Description
YDR055W	**21.3**	**5.8**	**PST1**	**Strong similarity to SPS2 protein**
YLR194C	21.1	6.8		Hypothetical protein
YJL153C	**17.9**	**5.7**	**INO1**	**Myo-inositol-1-phosphate synthase**
YHR209W	16.8	8.7		Similarity to hypothetical protein YER175c
YCR007C	15.0	4.2		Strong similarity to subtelomeric encoded proteins
YGR213C	**13.2**	**4.3**	**RTA1**	**Involved in 7-aminocholesterol resistance**
YER091C	12.8	5.4	MET6	Homocysteine methyltransferase
YGL121C	9.3	3.9	GPG1	Hypothetical protein
YJR073C	**9.2**	**3.3**	**OPI3**	**Methylene-fatty-acyl-phospholipid synthase**
YPL088W	9.2	4.3		Similarity to aryl-alcohol dehydrogenases
YOR208W	9.1	3.1	PTP2	Protein-tyrosine-phosphatase
YKL163W	8.9	5.9	PIR3	Member of the Pir1p/Pir2p/Pir3p family
YLR121C	7.9	4.9	YPS3	GPI-anchored aspartyl protease 3 (yapsin 3)
YKR091W	7.7	1.4	SRL3	Similarity to YOR083w
YBL049W	7.0	2.2	MOH1	Strong similarity to hypothetical protein
YGL205W	**6.9**	**2.6**	**POX1**	**Acyl-CoA oxidase**
YFR026C	6.9	7.7		Hypothetical protein
YHR138C	6.8	1.9		Hypothetical protein
YDR085C	6.8	1.9	AFR1	Involved in morphogenesis of the mating projection
YFL014W	6.6	6.5	HSP12	Heat-shock protein
YGL125W	6.4	2.2	MET13	Putative methylene tetrahydrofolate reductase
YNR066C	6.2	2.1		Strong similarity to Pep1p
YDR077W	**5.9**	**2.1**	**SED1**	**Abundant cell surface glycoprotein**
YIL117C	**5.8**	**1.2**	**PRM5**	**Similarity to hypothetical protein YNL058c**

Table 3
Functional categories and subcategories of induced genes

Functional category Functional subcategory	Number of genes	(%)*
Cell cycle and DNA processing (628 ORFs)	35	5.6
Cell cycle (451 ORFs)	28	6.2
DNA processing (251 ORFs)	8	3.2
Cell fate (427 ORFs)	24	5.6
Cell differentiation (382 ORFs)	22	5.8
Cell growth/morphogenesis (96 ORFs)	7	7.3
Cell rescue, defense and virulence (278 ORFs)	38	13.7
Detoxification (102 ORFs)	9	8.8
Stress response(175 ORFs)	31	17.7
Cellular communication (59 ORFs)	3	5.1
Cellular transport/transport mechanisms (495 ORFs)	9	1.8
Control of cellular organization (209 ORFs)	6	2.9
Energy (252 ORFs)	19	7.5
Metabolism (1066 ORFs)	84	7.9
Amino acid metabolism (204 ORFs)	25	12.3
C-compound and carbohydrate metabolism (415 ORFs)	32	7.7
Lipid, fatty-acid and isoprenoid metabolism (213 ORFs)	15	7.0
Nitrogen and sulfur metabolism (67 ORFs)	8	11.9
Nucleotide metabolism (148 ORFs)	8	5.4
Protein activity regulation (13 ORFs)	1	7.7
Protein fate (folding, modification, destination) (595 ORFs)	19	3.2
Protein synthesis (359 ORFs)	5	1.4
Protein with binding function (4 ORFs)	0	0.0
Cellular environment (199 ORFs)	14	7.0
Transcription (771 ORFs)	21	2.7
Transport facilitation (313 ORFs)	7	2.2
Transposable elements (116 ORFs)	0	0.0
Classification not yet clear-cut (115 ORFs)	7	6.1
Unclassified proteins (2399 ORFs)	141	5.9

*(Number of induced genes)/(total number of genes in the category) × 100.

3.3. Induction of genes involved in protein metabolism after growth under 30 MPa pressure

Pressure treatment caused induction of genes in the subcategory of "stress response" (Table 4), including genes involved in energy metabolism, such as *PAU* genes, oxidative stress, such as *GRX1* and *CCT1*, and heat shock response, such as *HSP12*, *HSP150*, *HSP26*, *SSE2*, and *HSP104*. The *PAU* genes are not well characterized but these genes are expected to be important to the yeast and some of them are likely to help the yeast cope with anaerobiosis [18]. *GRX1* and *CTT* 1 encode glutaredoxin [19] and cytoplasmic catalase T [20]. *HSP12*, *HSP150*, *HSP26*, *SSE2*, and *HSP104* contribute to protein metabolism as molecular chaperons or chaperon regulators [21]. Previous work has shown that following recovery from lethal pressure, the majority of induced genes were involved in protein metabolism [6]. The induction of *HSP104*, *HSP12*, and *HS26* was also observed after lethal pressure treatment [6]. However, lethal pressure treatment induced factors for protein degradation such as *UBI4* and related genes [6]. *UBI4* encodes ubiquitin and this and related genes play a role in protein degradation [22]. In contrast, a pressure of 30 MPa, which inhibited cellular growth, activated protein metabolism but not protein degradation.

3.4. Activation of membrane or lipid related metabolism under 30 MPa

In addition to stress response genes, yeast cells activate genes for the metabolism of C-compound, lipid, and amino acid related materials. Table 4 shows lists of induced genes in the subcategories of "lipid, fatty-acid and isoprenoid metabolism" and "amino acid metabolism". In "lipid, fatty-acid and isoprenoid metabolism", *INO1*, and *OPI3* were strongly induced (Table 4). These two genes are typically induced by detergents, oils, and membrane stabilizers [14]. The highly induced genes *PST1*, *RTA1*, *SED1*, and *PRM5* (Table 2 in bold) were also induced by stresses that affect membrane structure. These factors have been shown to contribute to membrane related functions (http://mips.gsf.de/). These results suggest that yeast cells grown under 30 MPa pressure activate genes related to membrane structure.

In "amino acid metabolism", the activation of methionine biosynthesis is clearly understood (Table 4). The induction of genes in "nitrogen and sulfur metabolism" also reflects the biosynthesis of methionine (data not shown). Methionine biosynthesis was also shown to be correlated with lipid biosynthesis [14].

To confirm the activation of genes for membrane structure, we compared mRNA levels for *INO1*, *OPI3*, *PST1*, *RTA1*, *SED1*, and *PRM5* from cells grown under 0.1 and 30 MPa using RT-PCR. In Fig. 2, the induction of *INO1*, *PST1*, and *SED1* by high pressure was seen with 20 cycles. *RTA1* and *POX1* were seen with 24 cycles, and *PRM5* with 30 cycles. These results support the activation of genes for membrane structures.

4. Discussion

In this paper, we studied the effect of a hydrostatic pressure of 30 MPa, which caused growth inhibition to yeast cells and increased cellular size and complexity of the inner cellular structure after decompression. We found that a pressure of 30 MPa induced the production of certain heat-shock proteins and activated genes controlling membrane structure. The increased cellular size and complexity may be due to the change in membrane structure. The activation of genes for membrane structure caused by 30 MPa pressure is consistent with the observed cellular transformation. The effect of pressure on tryptophan permease in the membrane is under intense study by Abe et al. [2,7,8]. These researchers found that hydrostatic pressure in the range of 15–25 MPa caused arrest of the cell cycle in G1 phase in an exponentially growing culture of a *S. cerevisiae* tryptophan auxotroph. The study proved that G1 arrest was due to damage to tryptophan permease, which resulted in tryptophan starvation. They suggested that hydrostatic pressure might affect the activity of tryptophan permease through changes in the lipid bilayer structure [8]. The genome-wide mRNA expression profiles and the behavior of tryptophan permease under a pressure that caused growth inhibition suggest that pressure affects membrane structure. The membrane structure of eucaryotic microorganisms is more complex than that of procaryotic microorganisms and may be the reason that eucaryotic microorganisms are more sensitive to hydrostatic pressure.

Results presented here also demonstrate that the response of yeast cells to non-lethal pressures in the range that cause growth inhibition differs from that caused by lethal pressures [6]. It is clear that molecular chaperons were induced under both conditions. In contrast to inhibitory conditions, lethal conditions induced genes for protein degradation [6], suggesting that yeast

Table 4
Induced genes in "stress response", "lipid, fatty acid and isoprenoid metabolism" and "amino acid metabolism" subcategories

Stress response			Amino acid metabolism			Lipid related metabolism		
Systematic name	Standard name	Fold	Systematic name	Standard name	Fold	Systematic name	Standard name	Fold
YOR208W	PTP2	9.1	YER091C	MET6	12.8	YJL153C	INO1	17.9
YKL163W	PIR3	8.9	YGL125W	MET13	6.4	YJR073C	OPI3	9.2
YFL014W	HSP12	6.6	YJL088W	ARG3	4.5	YGL205W	POX1	6.9
YDR077W	SED1	5.9	YIR017C	MET28	4.3	YFL014W	HSP12	6.6
YMR096W	SNZ1	5.1	YKL001C	MET14	4.3	YNL111C	CYB5	3.0
YHR030C	SLT2	4.9	YER069W	ARG5,6	3.9	YER044C	ERG28	3.0
YJL159W	HSP150	4.5	YJR010W	MET3	3.6	YOL108C	INO4	2.9
YNL160W	YGP1	4.5	YKR069W	MET1	3.4	YGR157W	CHO2	2.6
YBR072W	HSP26	4.3	YFR030W	MET10	3.3	YIL160C	POT1	2.6
YIL011W	TIR3	4.0	YMR250W	GAD1	3.1	YAR044W	OSH1	2.5
YKL164C	PIR1	3.4	YDR502C	SAM2	3.0	YGR060W	ERG25	2.5
YCL035C	GRX1	3.2	YDR253C	MET32	2.9	YML008C	ERG6	2.4
YPL223C	GRE1	3.1	YJR137C	ECM17	2.8	YPL231W	FAS2	2.3
YGR088W	CTT1	3.0	YLR303W	MET17	2.8	YNL012W	SPO1	2.3
YHL046C		3.0	YAL012W	CYS3	2.7	YPL175W	SPT14	2.3
YCR083W	TRX3	2.9	YPR167C	MET16	2.7	YLR260W	LCB5	2.3
YEL049W	PAU2	2.9	YLR092W	SUL2	2.7	YKR053C	YSR3	2.1
YKL161C		2.9	YJR078W	BNA2	2.6			
YJL223C	PAU1	2.7	YFR018C		2.6			
YLR461W	PAU4	2.6	YER081W	SER3	2.4			
YFL020C	PAU5	2.5	YHR112C		2.4			
YAR020C	PAU7	2.4	YBR213W	MET8	2.3			
YNR076W	PAU6	2.4	YER042W	MXR1	2.2			
YML016C	PPZ1	2.4	YKL218C	SRY1	2.2			
YOL161C		2.3	YLR180W	SAM1	2.2			
YER042W	MXR1	2.2	YDR054C	CDC34	2.1			
YFL059W	SNZ3	2.2						
YIR037W	HYR1	2.2						
YBR169C	SSE2	2.1						
YLL026W	HSP104	2.1						
YDR293C	SSD1	2.1						

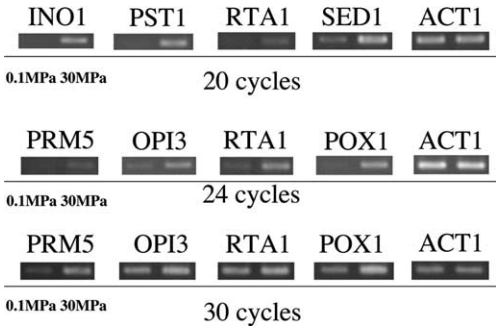

Fig. 2. Confirmation of the induction of genes as those that contribute to membrane structure. A RT-PCR was carried out using forward and reverse primers as described in Section 2. PCRs were carried out with 20, 24, and 30 cycles.

cells need to degrade denatured proteins. The inability to degrade the denatured proteins may result in cell death. However, it should be noted that the lethal response was a repair response after decomposition [6]. Kawano et al. showed that transcriptional activity of deep sea bacterium was not observed under 150 MPa [23], and transcription may not occur under lethal conditions of pressure. This is the reason why we monitored repair response for lethal conditions [6]. Recently, the response of yeast cells under pressure of 200 MPa was observed [24]; however under those conditions we cannot expect the new RNA synthesis, and the observed changes likely reflect the stability of the mRNA. On the other hand, the effect of pressure on membrane structure is significant under inhibitory conditions and yeast cells overcome this effect by activating the biosynthesis of membrane materials.

The most interesting range of pressures is that resulting in cellular arrest. This condition should be analyzed on a genome-wide basis or by using a model factor such as tryptophan permease. However, these experiments must be carefully designed such that mRNA synthesis and protein synthesis do not occur.

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